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CHEMICAL COMPOSITION AND MICROBIOLOGICAL PROPERTIES OF PELLETIZED CASSAVA BASED DIETS AT DIFFERENT DAYS OF STORAGE

B.O.ODUGUWA[1]*, O.A. ONI[2], A.A. ADEOLA[1]., S. A. FAMAKINDE[1], B. SANNI[3]., O.O. ODUGUWA[2]

[1]Livestock Production Research Programme, Institute of Food Security, Environmental Resources and Agricultural Research (IFSERAR), Federal University of Agriculture, Abeokuta, P.M.B. 2240, Abeokuta. Postcode 110001. Ogun State, Nigeria
[2] Department of Animal Nutrition, College of Animal Science and Livestock Production, Federal University of Agriculture, Abeokuta.
[3] Department of Animal Production and Health, University of Agriculture, Abeokuta
*__Corresponding author__: oduguwabo@funaab.edu.ng; **Tel:** +234803 211 0281

ABSTRACT

This study was carried out to determine storability, microbiological assay and chemical composition of pelletized cassava based diets. Four nitrogenous sources were used in the experimental diets as follows: T1, contained 200g/kg palm kernel cake (PK); 200g/kg caged layers droppings (CL) (T2), 200g/kg *Gmelina arborea* (GA) leaves (T3) and 40g/kg urea (UR)(T4). All the diets were pelletized. Other ingredients (g/kg) that made up each of the treatments were as follows: cassava sievate, 205; molasses, 100; Sulphur, 3; corn bran, 80; salt, 7; vit./min. Premix, 5. Media used were *Salmonella Shigella* agar, Potato dextrose agar, Manitol salt agar and Eosine methylene blue agar. Samples (1g) were taken at intervals of 1, 15 and 30 days for microbial analysis, while chemical composition was analysed at 1 day and 30 days after storage. Data generated were subjected to one way Analysis of Variance using Completely randomized design. No visible colour change, caking and mould growth were observed. Most of the bacteria and fungi isolated (*Staphylococcus aureus, Enterobacter* sp., *Mucour* sp., *Aspergillus* sp.,) were persistent throughout the storage period. There was no significant difference between microbial profile and total viable counts of the diets forms. GA had the highest level of crude protein. Proximate and fibre fractions of the diets reduced with increase in storage age. Thus, feeding animals with the pelletized cassava-based feed with urea as the non-protein nitrogen source either readily or after storage is recommended.

Keywords: Cassava, pelletizing, storability, properties, microbes, cattle.

INTRODUCTION

Feed accounts for about 70 percent of livestock production cost and it is the main factor limiting livestock productivity in Nigeria. Non-availability of animal feed is one of the greatest constraints to the expansion of the livestock industry in developing countries. During the dry season, there is shortage of forage for the ruminant animals to graze. Even then, the available forage is characterized by high fiber content, high cell wall content which include lignin and acid detergent fiber (ADF), low level of crude protein and minerals which result in to low level of feed

intake by the animals and drop in animals' growth and productivity (Oni *et al*., 2010).

Cereal grain, a major source of energy in livestock feed, is not readily available due to competition between man and monogastric animals. The cost of producing ruminants on concentrate supplement is very expensive and prohibitive to the resource poor farmers. Based on this, there is the need to develop cheaper sources of balanced nutrient blends during the dry season using available and cheaper non-conventional but novel feed ingredients.

The use of composite feed consisting of available and affordable feed resources such as cassava products fortified with different sources of protein, vitamins and minerals can prevent scarcity of nutritious feed for ruminants. Cassava roots and by-products offer tremendous potential as cheap alternative feedstuff to maize and other highly competitive and scarce sources of feed ingredients (Agunbiade *et al*., 2001). However, utilization of cassava in animal feed is limited by its short shelf life, low protein content and presence of anti-nutritional factors such as hydrocyanic acid (HCN). The reliability of cassava products and by-products will to a large extent depend on how well they can be processed and stored into safe consumable forms (Bokanga, 1995).

Simple processing techniques such as sun drying and milling will greatly improve the shelf life and reduce the level of cyanogens in cassava. The nutritional value of cassava could be improved by fortifying it with protein source such as Non Protein Nitrogen (NPN) and browse leguminous tree, which are rich sources of proteins and some minerals. Pelletising of feed is rare in ruminant animal production in Nigeria. However, pelletising of the feed would be highly beneficial to the animal. Behnke (1994) reported that pelletising of feed decreased feed wastage, reduced selective feeding, decreased ingredient segregation, destroyed pathogenic organisms, improved palatability and assisted in thermal modification of starch and protein. The study was designed to determine the physical, chemical and microbiological qualities as well as storage stability of pelletized cassava-based diets containing different nitrogenous sources for ruminants.

MATERIALS AND METHODS

Sources of materials

Whole cassava tubers and cassava peels were purchased from cassava processing outlets in Abeokuta, Nigeria. *Gmelina arborea* leaves were plucked from *Gmelina* multiplication unit of the Federal University of Agriculture, Abeokuta (FUNAAB), while the caged layer droppings were collected from the Teaching and Research farm of FUNAAB. Urea, salt, vitamins, bone meal and sulphur were purchased from a reputable feed mill.

Experimental diets preparation and design

The cassava tubers (unpeeled) were chipped and sun dried for five days to about 10% moisture content to facilitate milling as well as to reduce the toxicity of the hydrogen cyanide found naturally in it. The poultry droppings, cassava peels and the *Gmelina* leaves were also sun dried for easy milling. The dried cassava chips were packed in 50kg sacks at room temperature for four days to further reduce the hydrogen cyanide content before taking to feed mill for further processing. All the dried feed ingredients were milled and mixed with the vitamins and minerals into four different rations (Table 1). The four diets were thereafter pelletized.

Table 1: Composition of cassava based diets containing different nitrogen sources

Components	Treatments (%)			
	PK	CL	GA	UR
Dried cassava pulp	18	38	38	47
Dried cassava peel	-	40	40	47
Wheat offal	60	-	-	-
Caged layers droppings	-	20	-	-
Gmelina arborea leaves	-	-	20	-
Urea	-	-	-	4.0
Palm kernel cake	20	-	-	-
Sulphur	0.3	0.3	0.3	0.3
Common Salt	0.7	0.7	0.7	0.7
Vitamin /mineral premix	0.5	0.5	0.5	0.5
Bone meal	0.5	0.5	0.5	0.5
Total	100	100	100	100

PK- Palm kernel cake; CL- Caged layers droppings; GA- Gmelina arborea; UR- Urea

Chemical Analyses

Samples were also taken at 1st day and 30th day for the chemical analysis. Proximate compositions of the diets were determined according to A.O.A.C. (1990). The method of Hopkins *et al*. (1995) was used for Neutral Detergent Fibre (NDF) determination. The method of Saura-Calixto *et al*. (1983) was used for Acid detergent fibre (ADF) determination.

Data collection

Data were collected on microbiological assay as well as on chemical analysis during the storage period at different days.

Microbial analysis

Each of the diet was stored in a 25kg jute bag and stored at room temperature. Samples from each bag were taken at 1st , 15th and 30th day for microbial analysis. The microbial assay was carried out at the Department of Microbiology, FUNAAB.

Sterilization and preparation of growth media

The working surfaces were cleaned with methylated spirit soaked cotton wool to make the area sterile. MacCartney bottles containing 9mls distilled water to be used as diluents was sterilized in an autoclave at 121^{o}C for 15 minutes.

Growth media used were Nutrient Agar (NA), Mannitol salt agar (MSA), *Salmonella shigellae* agar (SSA), Eosine methylene blue agar (EMBA) and Potato dextrose agar (PDA). Each of the agars was weighed on the sensitive scale following manufacturers' description and dispensed into a 250mls conical flask, distilled water was added to each flask and placed into the water bath to dissolve the agar easily. The conical flask was cotton plugged and autoclaved for 15 minutes at 121^{o}C. Spirit lamp flame was used to achieve aseptic working environment during plating.

Serial dilution

One gram of each sample of the experimental diet was measured on the sensitive scale and suspended into MacCartney bottle containing 9mls sterile distilled water. 1ml of each sample was taken from MacCartney bottles with the aid of sterile pipette and placed in a Petri dish. Each sample was replicated five times in Petri dishes and each petri dish was labeled for identification purpose. Each of the growth media (NA, MSA, SSA, EMBA and PDA) was poured into a particular replicate of a sample in a Petri dish (plate) and covered.

Total bacterial count

The total bacteria count for each sample was determined with the pour plate technique using nutrient agar. The plates were incubated for 24 hours at 37^{o}C. All colonies that appeared at the end of the incubation period were counted using digital illuminated colony counter and the counts were expressed in colony forming unit per gram (CFU/g) of the sample. Colonies of bacteria developing on the plates were observed, isolated, and re-isolated on a fresh media until pure culture was obtained.

Identification of Isolates

Bacteria isolates were identified using morphological culture characteristics which are colour, consistency, shape, size, elevation, edge, opacity among others; biochemical tests such as citrate, coagulase motility, indole, etc. and sugar fermentation test. The fungi isolates were identified using cultural and morphological characteristics such as filamentous, rough, raised, conidiospore, sexual and non septate.

Data Analysis

All data collected were subjected to a Complete Randomized Design using Minitab Analytical computer package (Minitab Inc., 1989). Significant means were separated using Duncan's multiple range test (Duncan, 1955).

RESULTS AND DISCUSSION

Throughout the 30 days storage period, no visible colour change was observed in all the four diets, caking and mould growth were also not observed. Scudamore *et al*. (1997) reported the occurrence of mycotoxins in 330 samples of raw ingredients used for animal feeding in the 186 feedmills in the United Kingdom in 1992. They reported that no cassava sample was found to be contaminated with aflatoxin or other mycotoxins, while samples of maize, rice bran, cotton seed meal, palm kernel meal, wheat and barley were found to be contaminated with aflatoxin B1, fumonisin B1 and B2, ochratoxin A or Zearlone. Tables 2 and 3 showed the isolated microorganisms and their counts for different days of storage. As the length of storage increased, the isolated microorganisms increased in population. The microorganisms isolated during the periods of storage were mostly species of *Aspergillus, Staphylococcus, Saccharomyces* and *Mucor*. Most of these microorganisms are identified with feed spoilage which has a detrimental effect on the health of the animals that consume such feed. However, some beneficial importance of these microorganisms at a particular level had been studied and documented by some researchers. Direct fed microbials (DFM) have been used in ruminants to improve growth and reduce ruminal infection. Microorganisms used in DFM for ruminants include species of *Streptococcus*, *Bacillus* among others. Most commercial yeast products contain species of *Saccharomyces* and *Aspergillus. Saccharomyces cerevisiae* was able to compete with other starch utilizing bacteria for fermentation of starch (Lynch and Martin,

2002) leading to the prevention of lactate accumulation in the rumen. Chaucheyras *et al.* (1995) also reported that *Saccharomyces cerevisiae* had the ability to provide growth factors, such as organic acids or vitamins, thereby stimulating ruminal populations of cellulolytic bacteria and Lactobacillus utilizing bacteria (LUB) energetic efficiency and reducing ketosis (Weiss *et al.*, 2008). Jouany *et al*. (1999) observed a significant decrease in redox potential, up to -20 mV, in the rumen with yeast supplementation. This change creates better conditions for the growth of strict anaerobic cellulolytic bacteria, stimulates their attachment to forage particles (Roger *et al*., 1990), and increases the initial rate of cellulolysis. *Saccharomyces cerevisiae* at population of 3 x 10^9 cfu/flask/kg produce higher feed efficiency in calves (Malik and Bandla, 2010). Oetzel *et al*. (2007) reported that *E. faecium* plus *Saccharomyces cerevisiae* increased milk fat percentages when used as DFM for first lactation cows and increased milk protein percentages for second and greater lactation cows during the first 85 DIM. Second-lactation cows receiving DFM also received fewer antibiotic treatments. *Saccharomyces cerevisiae* at 5 x 10^9 cfu/cow/day first lactation cows fed DFM produced more milk fat % and second lactation cows fed DFM received fewer antibiotic treatments (Oetzel *et al*., 2007). Cows fed DFM had a higher live weight (Kowalski *et al*., 2009). Table 4 shows the chemical composition of cassava based diets, as the length of storage increased, all the proximate composition significantly reduced except for the acid detergent fiber which increased with storage. PK and UR had the highest level of crude protein at day 1 which were significantly different ($P < 0.05$), this may be due to their sources of protein supplement. However, urea diet was not significantly different at days 1 and 30. GA had the highest level of fibre which was significantly different from others, while PK and UR had the lowest level of crude fibre. The ADF increased with storage, this may reduce digestibility as the length of storage increased. According to ARC (1980), the maximum protein requirement for ruminant irrespective of their age or production level is 20% while the maximum energy level is 40% depending on the crude protein level in the diet.

Table 2: Microorganisms isolated from each diet at different days of storage

Days of storage	Parameters	Microorganisms isolated
1st day	PK	Staphylococcus aureus and Bacillus subtilis
"	CL	Staphylococcus aureus
"	GA	Staphylococcus aureus
"	UR	Staphylococcus aureus
15th day	PK	Staphylococcus aureus, Bacillus subtilis and Aspergillus flavus
"	CL	Staphylococcus aureus and Saccharomyces cerevisae
"	GA	Staphylococcus aureus, Aspergillus flavus and Mucor spp
"	UR	Staphylococcus aureus and Salmonella spp
30th day	PK	Aspergillus flavus, Enterobacter acrogens, Salmonella spp, Penicillium spp and Paeciliomyces spp
"	CL	Salmonella spp, Shigella spp, Enterobacter Aerogenes, Aspergillus flavus and Trichoderma spp
"	GA	Salmonella spp, Proteus spp, Trichoderma spp, Mucor spp
"	UR	Salmonella spp, Shigella spp, Enterobacter Acrogens, Aspergillus flavus and Aspergillus niger

PK- Palm kernel cake; CL- Caged layers droppings; GA- Gmelina arborea; UR- Urea

Table 3: Microbial count (CFU/ml) of the Experimental diets

		Media used				
Days	Parameters	NA (104)	SSA (103)	EMBA (104)	MSA (104)	PDA (102)
1st	PK	1.16	-	-	1.84	-
"	CL	1.33	-	-	1.76	-
"	GA	0.98	-	-	1.92	-
"	UR	1.20	-	-	1.72	-
15th	PK	2.08	-	-	2.16	3.00
"	CL	2.11	-	-	2.22	22.00
"	GA	2.02	-	-	2.34	2.00
"	UR	1.66	-	-	2.21	21.50
30th	PK	2.82	2.20	2.17	1.04	21.00
"	CL	2.40	4.60	2.08	1.86	8.00
"	GA	2.60	18.60	2.74	1.74	11.00
"	UR	2.32	8.50	2.82	1.65	8.00

CFU/ml=Colony forming unit per mil
NA= Nutrient agar; MSA= Mannitol salt agar
SSA= Salmonella shigellae agar; EMBA=Eosine methylene blue agar
PDA= Potato dextrose agar

PK- Palm kernel cake; CL- Caged layers droppings; GA- Gmelina arborea; UR- Urea

Table 4: Chemical composition of cassava based diet (%) at 0 and 30 days of storage

					30 days of storage				
Parameters	PK	CL	GA	UR	PK	CL	GA	UR	SEM
CP	23.42a	22.77b	20.42f	24.52a	22.50c	20.77c	21.55d	23.89a	0.17
EE	2.63a	2.44cd	2.53b	2.73d	2.59a	2.40d	2.46c	2.68a	0.15
Ash	9.35c	10.02b	10.22a	8.43cd	9.32c	9.88b	10.00b	8.41c	0.05
CF	11.66c	11.78b	11.88a	11.15c	8.86f	9.97d	9.92c	9.02a	0.01
DM	90.48d	90.55c	90.66b	90.32a	90.52cd	91.00a	90.95a	90.88a	0.02
NFE	42.46c	242.44c	44.29a	40.75b	42.19b	42.49c	44.32a	40.34d	0.04
ADF	50.99c	51.11b	50.70c	50.65c	51.12b	52.08a	50.77d	50.71c	0.02
NDF	35.01c	34.22d	35.91a	34.86c	35.33b	34.15d	35.88a	34.65b	0.03

abcde means on the row with different superscripts are significantly different (P<0.5)
SEM=Standard Error of Means
CP=Crude Protein, CF=Crude Fibre, EE=Ether Extract, DM=Dry Matter
NFE=Nitrogen Free extract, ADF=Acid Detergent Fibre, NDF=Neutral Detergent Fibre
PK- Palm kernel cake; CL- Caged layers droppings; GA- Gmelina arborea; UR- Urea

CONCLUSION

The absence of any physical change in terms of colour and no mould growth for the period of 30 days of storage mean that the feed would still be both acceptable and palatable to animals after 30 days of processing and also safe for their consumption. The microbial counts throughout the length of storage were within the tolerance level for safe consumption of ruminant animals. Most of these microorganisms at this level rather than being harmful were beneficial for livestock as most of them had been used in direct fed microbial studies. The reduced level of these microorganisms and can be attributed to low moisture content before processing the feed and also to processing method adopted. The level of crude protein in the feed was within the acceptable range, even though it reduced with storage.

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